REMARKS

Claims 69-135 are currently pending in the application. Claims 120-135 have been withdrawn from consideration by the Examiner as being drawn to a non-elected invention. Claims 104, 105, and 107-116 have been withdrawn from consideration by the Examiner as being drawn to a non-elected species. Claims 69-103, 106, and 117-119 are currently under examination.

As noted above, the Applicant understands claim 103 to remain under consideration. In the current Office Action mailed on 9 April 2007, the Office Action Summary indicates that claim 103 is under consideration and is currently rejected. The Detailed Action, however, is not consistent in its treatment of claim 103. In the Detailed Action, on page 2, numbered paragraph 1, the Examiner indicates that claim 103 has been withdrawn from further consideration. The Applicant understands this to be a simple typographical error, but the Applicant would appreciate receiving clarification from the Examiner.

In support of Applicant's understanding that claim 103 remains under consideration, the Applicant notes that in the current Office Action mailed on 9 April 2007, on page 2, numbered paragraph 2, the Examiner indicates that the individual species claims are claims 104-116. The Applicant also notes that in the Restriction/Election Requirement mailed on 5 February 2007, the Examiner indicated that claims 104-116 were considered to be species claims.

Declaration

The Examiner notes that the Declaration is defective because it does not contain the mailing address of the inventor. The Applicant thanks the Examiner for pointing out the deficiency in the Declaration. The original Declaration as filed was two (2) pages with the Applicant's address and signature on the second page. In PAIR, however, only the first page of the original Declaration is available. It appears that the first page, but

not the second page, of the original Declaration was scanned into PAIR. A copy of the original two (2) page Declaration is being filed with this Amendment and Response.

Claim Amendments

Claim 74 has been amended to define the acronym IMDM as "Iscove's modified Dulbecco's medium." Support for this amendment is found in the original specification as filed in the listing of abbreviations which states "IMDM, Iscove's modified Dulbecco's medium" at page 17, lines 19-20. Additional support for this amendment is found at page 22, lines 14-15, and page 22, lines 23-24, where commercial sources of Iscove's modified Dulbecco's medium are also provided.

Interview Summary

The Applicant and the Applicant's Representative thank the Examiner for the personal interview conducted with the Examiner at the U.S. Patent and Trademark Office on 21 August 2007. The Applicant and the Applicant's Representative greatly appreciate the Examiner's time, interest, and consideration. The Examiner prepared an Interview Summary dated 21 August 2007 which the Applicant accepts as an accurate summary of the substance of the interview. Much of the content of the interview will also be addressed in this Amendment and Response in greater detail.

The Applicant notes that the interview summary refers only to the parent application (U.S. Patent Application Serial No. 10/059, 521) of the instant application because the issues were discussed in the context of the parent application. Nonetheless, the same issues are relevant to the present application, and the claims of the present application stand rejected on almost identical grounds.

Substance of Interview

During the Interview on 21 August 2007, the Applicant explained how the claimed invention is fundamentally different from the prior art. The Examiner has generally relied on the combination of (a) a prior art colony forming assay and (b) an ATP bioluminescence assay to provide the basis for an obviousness rejection under 35 U.S.C. § 103(a). The Applicant explained that the claimed invention is not merely a novel combination of elements found in the prior art. The claimed invention is, in fact, fundamentally different from any of the cited art, either alone or in combination.

Proliferation is the process by which cells increase in number by division to produce daughter cells identical to the parent cell. Differentiation is a process by which unspecialized cells develop the structures and functions characteristic of a particular mature cell type. In the case of stem cells, a stem cell can proliferate to form additional stem cells. Alternatively, a stem cell can further differentiate into committed cells and differentiated cells.

The traditional colony forming assay (CFA) found in the prior art is a differentiation assay which requires manual counting of differentiated colonies, generally after 14 days in culture. The cells are allowed to differentiate and form colonies of functionally mature cells so they can then be identified morphologically according to colony type and counted manually. In contrast, the claimed invention is directed to a proliferation assay. The claimed invention provides a high throughput assay method for rapidly determining the proliferative status of a population of cells. While proliferation and differentiation are related, they are fundamentally different processes. Proliferation is required for the process of differentiation to occur, but differentiation is not required for the process of proliferation to occur. To the Applicant's knowledge, the claimed invention provides the first method which allows determination of the proliferative status of a population of cells and the first method which allows the process of proliferation to be separated out from the process of differentiation.

These issues will be addressed in greater detail in the context of the pending rejections and the cited prior art references.

Claim Rejections

A. Rejection of claims 69-103, 106, and 117-119 under 35 U.S.C. § 112, second paragraph, as being indefinite

The Examiner has rejected claims 69-103, 106, and 117-119 under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention.

1. Rejection of claim 69 under 35 U.S.C. § 112, second paragraph, as being indefinite in reciting "serum mix"

The Examiner asserts that claim 69 is indefinite in reciting "serum mix" because "it is unclear what is encompassed in reciting 'mix' which appears to be a term that lacks comparative basis for defining its metes and bounds." Office Action dated 9 April 2007, page 4.

The Applicant would like to call to the Examiner's attention the portions of the specification which define the term "serum mix." The specification explains that the preferred composition of the serum mix includes bovine serum albumin, insulin, transferrin, and IMDM. The application states:

> By identifying reduced serum conditions which support the expansion of many target cells and lineages, the assay staging pre-combines serum and serum replacement components, such that addition of a "serum" cocktail in a single standardized volume, compatible with the same repeating syringe dispensers described above, results in the desired concentrations of each component in each final plating mixture being assembled. This assay component is designated BITSI. Its preferred

> composition is Bovine serum albumin (50 mg/ml), recombinant human Insulin (50 µg/ml) iron-saturated Transferrin (1 mg/ml), Serum, and IMDM, although it is contemplated that the amounts and final concentrations of the individual components of BITSI may be varied in accordance with the requirements of a particular cell line being cultured. Application as filed, page 34, line 26 - page 35, line 6, emphasis added.

The term "mix" is used to describe both the serum mix and the methyl cellulose mix throughout the section entitled "HALO-related method kits" in the originally filed application at pages 33-38. As noted in the paragraph quoted above, the "serum mix" is also referred to as BITSI, and this component is referred to as a "mix" throughout the application. The application states:

> The kits of the present invention, therefore, provide lineage-specific plating mixtures from three premixed reagent mixes, each mix preferably provided in separate vessels (i.e. 2.5 x methyl cellulose, a 1.75% methyl cellulose base may contain 2.5 x final concentration of α-thioglycerol, BITSI reagent, growth factor cocktails).
> Application as filed, page 36, lines 4-8, emphasis added.

The terms "serum mix" or "serum component mix" are used to discuss the kit components and their use at page 43, lines 12-20, and in Example 13 at pages 61-64.

2. Rejection of claim 69 under 35 U.S.C. § 112, second paragraph, as being indefinite in reciting "methyl cellulose mix"

The Examiner asserts that claim 69 is indefinite in reciting "methyl cellulose mix" because "it is unclear what is encompassed in reciting 'mix' which appears to be a term

that lacks comparative basis for defining its metes and bounds." Office Action dated 9 April 2007, page 4.

The Applicant would like to call to the Examiner's attention the portions of the specification which define the term "methyl cellulose mix." The specification explains that the methyl cellulose mix preferably comprises methyl cellulose and α-thioglycerol. The application states:

> The kits of the present invention, therefore, provide lineage-specific plating mixtures from three premixed reagent mixes, each mix preferably provided in separate vessels (i.e. 2.5 x methyl cellulose, a 1.75% methyl cellulose base may contain 2.5 x final concentration of α-thioglycerol, BITSI reagent, growth factor cocktails).
> Application as filed, page 36, lines 4-8, emphasis added.

The application also refers to the methyl cellulose mix as preferably comprising methyl cellulose and α-thioglycerol (α-TG) in other instances. The application states:

> Purely in terms of liquid handling, the HALO procedure comprises the following stages: (i) assembly of lineage-specific plating mixtures from liquid components, i.e. BITSI, growth factor cocktails, target cells and medium; (ii) addition of methyl cellulose/α-TG mix using positive displacement liquid handling devices; (iii) distributing of aliquots, for example, 100 µl, of each plating mixture onto multi-well plates using positive-displacement liquid handling devices; (iv) addition of ATP releasing reagent to each of the culture wells; (v) addition of ATP luminescence-monitoring reagent to each of the culture wells; and (vi) addition of ATP-CR to each of the culture wells if apoptosis is to be measured.
> Application as filed, page 36, lines 17-25, emphasis added.

> Aliquots of a 2.5 x methyl cellulose/α-TG mix may be added manually to these plating mixtures, using a repeater pipette and a disposable positive-displacement syringe tip.
> Application as filed, page 37, lines 22-24, emphasis added.

The term "mix" is used to describe both the serum mix and the methyl cellulose mix throughout the section entitled "HALO-related method kits" in the originally filed application at pages 33-38. The term "methyl cellulose mix" is also used to discuss the kit components and their use at page 43, lines 12-20, and in Example 13 at pages 61-64.

3. Rejection of claim 69 under 35 U.S.C. § 112, second paragraph, as being indefinite in reciting "a mix of growth factors" and "a mix of cytokines"

The Examiner asserts that claim 69 is indefinite in reciting "a mix of growth factors" and "a mix of cytokines" because "it is unclear what is encompassed in reciting 'mix' which appears to be a term that lacks comparative basis for defining its metes and bounds." Office Action dated 9 April 2007, page 4.

The term "mix," when used in the terms "a mix of growth factors" and "a mix of cytokines" clearly takes on its plain and ordinary meaning, in this case referring to a combination of growth factors or a combination of cytokines. Claim 69 recites a proliferation agent, "the proliferation agent selected from the group consisting of a single growth factor, a mix of growth factors, a single cytokine, a mix of cytokines, and combinations thereof." This could be rephrased as "one or more growth factors, one or more cytokines, and combinations thereof."

Examples of "a mix of growth factors" and "a mix of cytokines" are present throughout the application. The application explains:

> Combinations of growth factors may be used to stimulate the proliferation of each lineage tested using the HALO procedure. For example, 6 different growth factors are required to stimulate the proliferation of CFC-GEMM, and three different growth factors are required to stimulate the proliferation of BFUe, GM-CFC and Mk-CFC. Each of the growth factors required for a particular lineage can be combined, in appropriate proportions, into a lineage-specific growth factor mix, thereby obviating the need to add each of the required growth factors separately. A combination of IL-3, IL-6, SCF, GM-CSF, EPO, and G-CSF, added respectively at, for example, doses of 1, 2, 3, 4, 10 and 20 ul per ml of plating mixture, can be used to stimulate the proliferation of CFC-GEMM. 40 µl of this GEMM-specific cocktail would be required for each 1 ml of lineage-specific plating mixture being prepared. Similarly, for BFU-E, IL-3, SCF, and EPO, respectively at doses of 1, 3 and 10 ul per ml of plating mixture, could be premixed and 14 µl of the resulting BFU-E-specific cocktail would be required per ml of plating mixture being prepared. Also, for example, 8 µl of a 1:3:4 premix of IL-3, SCF, and GM-CSF is used to stimulate the proliferation of GM-CFC, and 5 µl of a 1:2:2 premix of IL-3, IL-6 and TPO would be used to stimulate the proliferation of Mk-CFC. Large volumes of pre-mixed growth factor combinations are provided by the kits of the present invention, aliquoted, and frozen ready for use.
>
> Application as filed, page 35, line 15 - page 36, line 3, emphasis added.

The term "mix" is used to describe combinations of growth factors and cytokines throughout the section entitled "HALO-related method kits" in the originally filed application at pages 33-38. The term "growth factor mix" is also used to discuss the kit components and their use at page 43, lines 12-20, and in Example 13 at pages 61-64. Specific examples of growth factor and cytokine combinations that may be particularly useful in various embodiments of the present invention are described at page 29, line 6 - page 30, line 23, and in Example 3 and Table 3 at page 51, line 23 - page 52.

The Applicant would also like to address the use of the term "proliferation agent" in claim 69. The Examiner has raised no objection to the use of the term "proliferation agent" in claim 69 in the instant Office Action. The Examiner did, however, request an explanation of support for the use of the term "proliferation agent" in the personal interview granted on 21 August 2007. The interview summary refers only to the parent application (U.S. Patent Application Serial No. 10/059, 521) of the instant application because the issues were discussed in the context of the parent application. Nonetheless, the same issues are relevant to the present application, and the claims of the present application stand rejected on almost identical grounds.

Claim 69 recites, in relevant part, "a proliferation agent specific for a single subpopulation within the target cell population of mononuclear cells, the proliferation agent selected from the group consisting of a single growth factor, a mix of growth factors, a single cytokine, a mix of cytokines, and combinations thereof." Numerous specific examples of growth factor and cytokine combinations that may be useful in various embodiments of the present invention are described at page 29, line 6 - page 30, line 23, and in Example 3 and Table 3 at page 51, line 23 - page 52.

The term "proliferation agent," as used in claim 69, is defined as "a single growth factor, a mix of growth factors, a single cytokine, a mix of cytokines, and combinations thereof." Support for use of the term "proliferation agent" appears throughout the application and specifically on page 15, lines 2-17 of the application as filed. Additional

examples of one or more cytokines and/or growth factors use as a "proliferation agent" include the following:

> The terms "modulating the proliferative status" or "modulating the proliferation" as used herein refers to the ability of a compound to alter the proliferation rate of a population of hematopoietic stem or progenitor cells. A compound may be toxic wherein the proliferation of the cells is slowed or halted, or the proliferation may be enhanced such as, for example, by the addition to the cells of a cytokine or growth factor.
> Application as filed, page 13, lines 16-21, (emphasis added).

> Such proliferation enhancing compounds include, for example, cytokines and growth factors.
> Application as filed, page 21, lines 12-13, (emphasis added).

Applicant believes that the term "a proliferation agent, the proliferation agent selected from the group consisting of a single growth factor, a mix of growth factors, a single cytokine, a mix of cytokines, and combinations thereof" is adequately supported by the application as filed.

4. Rejection of claim 74 under 35 U.S.C. § 112, second paragraph, as being indefinite in reciting "IMDM"

The Examiner asserts that claim 74 is indefinite in reciting "IMDM" because IMDM is an acronym. Office Action dated 9 April 2007, page 4. Claim 74 has been amended to define the acronym IMDM as "Iscove's modified Dulbecco's medium." Support for this amendment is found in the original specification as filed in the listing of abbreviations which states "IMDM, Iscove's modified Dulbecco's medium" at page 17, lines 19-20. Additional support for this amendment is found at page 22, lines 14-15, and

page 22, lines 23-24, where commercial sources of Iscove's modified Dulbecco's medium are also provided.

5. Rejection of claim 77 under 35 U.S.C. § 112, second paragraph

The Examiner asserts that claim 77 is confusing in relation to the claims from which it depends, particularly in the use of the terms "fetal bovine serum" in part (a) and "methyl cellulose" in part (b). The terms "fetal bovine serum" in part (a) and "methyl cellulose" in part (b) refer to final concentrations of those components in an assay system. This is on contrast to the "serum mix" in claim 74 and the "methyl cellulose mix" in claim 76. As described in the application, the concentrations of components in the various mixes would be greater than their resulting concentrations in the final assay.

B. Rejection of claims 69-73 under 35 U.S.C. § 103(a) as being unpatentable over Bell et al., U.S. Patent Application Publication No. US 2002/0120098 A1, in view of Crouch et al., *Journal of Immunological Methods*, 160: 81-88 (1993)

Claims 69-73 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Bell et al., U.S. Patent Application Publication No. US 2002/0120098 A1, in view of Crouch et al., *Journal of Immunological Methods*, 160: 81-88 (1993). Applicant respectfully submits that none of the cited references, either alone or in combination, render the claimed invention obvious. Applicant requests that the Examiner withdraw these rejections in view of the remarks presented herein.

As stated in the MPEP:

> To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success.

> Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art and not based on applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).
> MPEP 706.02(j), emphasis added.

Applicant submits that the Examiner's rejection of the pending claims is improper because the Examiner has failed to provide a clear suggestion or motivation that would have led one of ordinary skill in the art to combine or to modify the prior art references in the manner suggested by the Examiner. More importantly, even if the prior art references were combined as the Examiner suggests, the combination would not result in the claimed invention because the combined references do not teach or suggest all of the claim limitations as required for a proper 35 U.S.C. § 103(a) rejection.

The claimed invention is directed to a system for assaying the **proliferative state** of a population of mononuclear cells. In simple terms, the Examiner relies on (1) Bell et al. for teaching various tissue culture conditions, and (2) Crouch et al. for teaching an ATP bioluminescence assay. None of the prior art references, however, teach or suggest a system for assaying the **proliferative state** of a population of mononuclear cells. Accordingly, the Applicant submits that the pending claims are patentable over all art cited and of record.

Bell et al.

The Examiner notes that Bell et al. teach similar culture conditions to those recited in the rejected claims. The Examiner asserts that "Bell et al. differ from the instant invention in failing to disclose a reagent capable of generating luminescence in the presence of ATP. The pending claims, however, are directed to a system for assaying hematopoiesis and hematotoxicity in which it is possible "to **determine the proliferative state** of the single subpopulation by luminescence output thereof."

In contrast to the claimed invention, Bell et al. is directed to a differentiation assay. Bell et al. explains:

> [0084] The Detection of Erythroid Progenitors in the **Colony Formation Assay**
>
> [0085] Hemoglobin enhances the growth of erythroid progenitors, notably the BFU-E progenitor population as shown by a **well-known technique** in the field of erythropoiesis research. This technique, referred to as the **colony formation assay (CFA)**, is the most widely used biological assay to identify and enumerate erythroid progenitors present in hematopoietic tissue such as blood and bone marrow. Cell populations containing erythroid progenitors are plated in semi-solid suspensions of methyl cellulose, agar, low melting agarose or related substances in nutrient culture medium containing 1-3 U/ml Epo and 10 ng/ml IL-3 to specifically stimulate erythroid progenitors. **Plates are incubated at 37° C. for 14 days during which time the erythroid progenitors form characteristic hemoglobinized (red) colonies**. **Two distinct but related erythroid progenitors can be distinguished based on colony size and morphology**: BFU-E, the most primitive recognizable erythroid

> progenitor forms large multilobular colonies whereas the more mature CFU-E forms smaller spherical colonies. Under these conditions other myeloid progenitors, notably the colony forming unit--granulocyte-macrophage (CFU-GM), also form morphologically identifiable non-hemoglobinized (white) colonies which are readily distinguished from erythroid colonies. Additional cytokines, notably IL-1, IL6, stem cell factor, flt-3 ligand, and granulocyte-macrophage colony stimulating factor, may be included in the CFA to stimulate both erythroid and nonerythroid progenitor proliferation.
>
> Bell et al., page 9, paragraphs [0084] - [0085], emphasis added.

This passage from Bell et al. makes it clear that the traditional colony forming assay of the prior art was used by Bell et al. It is also clear that the colony forming assay used by Bell et al. is a differentiation assay. In the procedure used by Bell et al., the plates must be incubated for 14 days in order for the cells to differentiate into mature cell types. After the cells have differentiated, the colonies are identifiable based on the morphology of the differentiated cell type.

The Examples in Bell et al. also disclose only a colony forming assay, or differentiation assay. For example, Examples 1 and 2 of Bell et al. state the following:

> The LDMNC were then plated into **colony formation assays (CFA)** on the second day of isolation.
>
> Bell et al., page 14, Example 1, paragraph [0121], emphasis added.

> Unless indicated otherwise, **under standard CFA conditions ...**
>
> Bell et al., page 14, Example 1, paragraph [0121], emphasis added.

> The number of hematopoietic progenitors was scored **between days 13-15 by counting the number and types of colonies present**.
>
> Bell et al., page 14, Example 1, paragraph [0122], emphasis added.

> Erythroid progenitor **colony formation assays were conducted as described in Example 1 ...**
>
> Bell et al., page 15, Example 2, paragraph [0124], emphasis added.

Unlike the claimed invention, Bell et al. disclose only the traditional colony forming assay of the prior art. The colony forming assay is a differentiation assay in which cells are allowed to differentiate and form colonies or clusters. Once the cells have differentiated into mature cell types, the colonies can be identified morphologically and counted manually.

Crouch et al.

The Examiner asserts that:

> "Crouch et al. disclose a system and instructions for determining the proliferative status (cell proliferation) of a population of primitive (lymphoblastic, promyelocytic) hematopoietic cells. The hematopoietic cells are granulocyte-macrophage colony-forming cells (GM-CFC) and granulocyte colony-forming cells (G-CFC), i.e. TF-1 and NSF-60 cells." Office Action, mailed 9 April 2007, page 7.

The Applicant respectfully disagrees with the Examiner's understanding of Crouch et al. Crouch et al. do not, in fact, disclose a system for determining the proliferative status of primitive hematopoietic cells. Crouch et al. do not even disclose the use of any primitive hematopoietic cells for any purpose. Applicant suggests that the confusion may have arisen because Crouch et al. discusses the use of granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF). Perhaps the Examiner may have confused the growth factors used in Crouch et al. with cell types that are not disclosed in Crouch et al.

The cells that are used in Crouch et al. are not primitive hematopoietic cells. On the contrary, the cell types to which the Examiner refers (TF-1 and NFS-60 cells) are actually transformed cell lines, not primitive hematopoietic cells. Unlike primitive hematopoietic cells, transformed cell lines are in a state unregulated growth in culture, much like an *in vitro* tumor. According to the American Type Culture Collection (ATCC, at www.atcc.org), the TF-1 cell line was derived from an erythroblast from a human patient with erythroleukemia. The ATCC comments further state that "[t]he TF-1 cell line was established by T. Kitamura, et al. in October 1987 from a heparinized bone marrow aspiration sample from a 35 year old Japanese male with severe pancytopenia." Also according to the ATCC, the NFS-60 cell line was derived from a lymphoblast from a mouse with myelogenous leukemia. The ATCC comments further state that "[t]he M-NFS-60 cell line was derived from a myelogenous leukemia induced with the Cas-Br-MuLV wild mouse ecotropic retrovirus."

While Crouch et al. does disclose an ATP bioluminescence assay, Crouch et al. does not teach or suggest an assay for determining the proliferative state of a population of mononuclear cells. Nor does Crouch et al. teach or suggest the claimed cell growth medium.

The Claimed Invention

The claimed invention provides a system for assaying hematopoiesis and hematotoxicity in a population of mononuclear cells by determining the proliferative state of the single subpopulation by luminescence output thereof.

The present application explains:

> **The high-throughput stem/progenitor cell assay (HT-SPCA) of the present invention does not count colonies or differentiate between colony types. Rather, the HT-SPCA of the present invention measures the proliferation status of cells within the colonies by determining the amount of ATP being produced by the cells**. With colony growth in the methyl cellulose assay system of the present invention, some cells in the cultures will begin to proliferate and form aggregates or clusters. However, the proliferative status of the cell population may be limited due to their late stage of differentiation. Thus, a small colony may ensue within a short incubation period, but cell proliferation will rapidly cease.
> Application as filed, page 25, line 29 - page 26, line 7, emphasis added.

The present application also contains data evidencing some of the differences between the claimed invention and the prior art. Specifically, Example 4, on pages 53-54, and Figures 7 and 8 show that the claimed assay is measuring a different parameter than the colony forming assay found in the prior art. Example 4 compares the data obtained from a colony forming assay with the data obtained from the proliferation assay of the claimed invention.

The discussion in Example 4 explains:

> EXAMPLE 4 : Proliferation of Hematopoietic Stem and Progenitor Cells Measured by Colony Counting and ATP Determination
>
> When cell proliferation was measured as a function of time in culture, some aggregates or colonies contained cells that were proliferating, while others were not, as shown in FIGS. 7A-7C. Wells, therefore, could contain few colonies, but still exhibit high cell proliferation. The results shown in **FIGS. 7A-7C show that the number of cell clusters counted per well does not correlate with the cell proliferation** as detected using the luminescence of the present invention.
>
> In contrast, in those wells in which minimal or no cluster formation was detected, luminescence could be detected. **In some wells, the luminescence was significantly greater than expected from the number of cell clusters counted, indicating that cell proliferation was occurring and that the proliferating cells, were primitive because of their increased proliferative capacity**. On day 10, most wells contained cells that were proliferating. By day 14, this proliferative capacity was only seen in some wells, indicating that proliferation has ceased (RLU lower than the cell cluster count) or is declining. **Those wells exhibiting a significantly greater RLU than determined by manual cell cluster counting showed that cells were present that were capable of extensive proliferation and were probably stem cells**.
>
> Little or no correlation existed between the number of individual colonies and the luminescence, as shown in FIGS. 8A-8C.
> Application as filed, page 53, lines 1-26, emphasis added.

As explained in Example 4 and illustrated in Figure 7, the number of cell clusters counted per well does not correlate with the relative luminescence per well. The reason for the lack of correlation is because the colony forming assay is a differentiation assay, whereas the claimed assay is a proliferation assay.

Figures 7 and 8 illustrate these results graphically as explained in the Brief Description of the Drawings:

> FIGS. 7A-7C illustrate histograms showing the number of cell clusters counted manually per well and the relative luminescence units (RLU) per well at day 7 (FIG. 7A), day 10 (FIG. 7B) and day 14 (FIG. 7C) of incubation.
>
> FIGS. 8A-8C graphically illustrate the lack of correlation between cell cluster counts per well and the relative luminescence units (RLU) per well on day 7 (FIG. 8A), day 10 (FIG. 8B) and day 14 (FIG. 8C) of culture incubation.
>
> Application as filed, page 9, lines 18-24.

In Figure 7, the solid bars represent the number of manually counted clusters per well under conditions of a traditional colony forming assay. These solid bars show the number of mature differentiated clusters in each well. The hatched bars represent the relative luminescence per well and reflect the level of cell proliferation occurring in each well. These two parameters do not correlate well because one is measuring differentiation and the other is measuring proliferation. Where the hatched bar is much higher than the corresponding solid bar for the same well, this means that relatively few numbers of clusters are producing a relatively high level of luminescence. This is an indication that the cells in that particular well are proliferating at a very high rate and are

likely stem cells. The claimed assay makes it possible to determine the proliferative status of a population of primitive cells and to identify stem cells within that population.

C. Rejection of claims 74-102, 106, and 117-119 under 35 U.S.C. § 103(a) as being unpatentable over Bell et al., in view of Crouch et al., and further in view of Tang et al., U.S. Patent No. 6,824,973.

Claims 74-102, 106, and 117-119 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Bell et al., U.S. Patent Application Publication No. US 2002/0120098 A1, in view of Crouch et al., *Journal of Immunological Methods*, 160: 81-88 (1993), and further in view of Tang et al., U.S. Patent No. 6,824,973.

The Examiner relies on Tang et al. to provide a tissue culture medium containing insulin and transferrin. The discussion of Bell et al. and Crouch et al. above applies equally to the present rejection. The combination of Bell et al. and Crouch et al. does not provide each and every element of the claimed invention. The addition of Tang et al. to the combination of references does not address the lack of a *prima facie* case of obviousness.

Since the Examiner has failed to established a *prima facia* case of obviousness with respect to independent claim 69, all claims depending from independent claim 69 are, therefore, also nonobvious. As stated in the MPEP:

> To establish *prima facie* obviousness of a claimed invention, all the claim limitations must be taught or suggested by the prior art. *In re Royka*, 490 F.2d 981, 180 USPQ 580 (CCPA 1974). ... If an independent claim is nonobvious under 35 U.S.C. 103, then any claim depending therefrom is nonobvious. *In re Fine*, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988). MPEP § 2143.03, emphasis added.

Conclusion

In summary, the combination of cited references do not yield the claimed invention, nor render the claimed invention obvious. For at least these reasons, Applicant respectfully requests that the Examiner withdraw the 35 U.S.C. § 103(a) rejection of the pending claims.

The art of record is directed to standard tissue culture media and traditional colony forming assays. Traditional colony-forming assays measure the ability of a primitive cell to differentiate into colonies, and are, therefore, differentiation assays. The present invention is directed to a system for determining the proliferative status of a population of cells. In contrast to the colony forming assays, or differentiation assays of the past, the present invention provides a proliferation assay. None of the cited art provides the motivation, suggestion, or teaching for a proliferation assay for mononuclear cells.

Any amendments made during the prosecution of this application are intended solely to expedite prosecution of the application and are not to be interpreted as acknowledgement of the validity of any rejection raised earlier in prosecution, nor as acknowledgement that any citation made against the application is material to the patentability of the application prior to amendment.

This Amendment and Response is being filed with a Request for Extension of Time for two (2) months under 37 C.F.R. § 1.136(a)(1) and the appropriate fee set forth in 37 C.F.R. § 1.17(a). No additional fees are believed necessitated by the filing of this Amendment and Response. Should any such additional fees be required, the Director is hereby authorized to deduct them from Deposit Account No. 18-2000, of which the undersigned is an authorized signatory.

Should the Examiner believe that there are any outstanding matters capable of resolution by a telephone interview, the Examiner is encouraged to telephone the undersigned attorney of record. Finally, the Applicant and the Applicant's Representative again express their appreciation for the Examiner's time and consideration during the personal interview on 21 August 2007.

Respectfully submitted

Donna E. Becker

Donna E. Becker
Reg. No. 44,529

10 September 2007

ROSENBAUM & ASSOCIATES, P.C.
650 Dundee Road
Suite #380
Northbrook, Illinois 60062
Tel. (847) 770-6000
Fax. (847) 770-6006

Docket No. 6115-002